

# EXPERIMENTAL STUDIES ON THE NUPTIAL PADS OF MALE TRITURUS VIRIDESCENS

CHARLES MARC POMERAT

(From the Biological Institute, Harvard University, and the Department of Biology,  
Clark University)

During the breeding season the adult male *Triturus viridescens* develops heavy black nuptial pads on the inner aspect of the femoral area and toe tips. There is also a marked hypertrophy of the tail fin, swelling of the cloaca, and development of the hedonic glands (Hilton, 1902). Adams (1932) reported the experimental production of leg-pad development, tail-fin response as well as ovulation and moulting following the administration of anterior pituitary extract (*Phyone*) in this species. Increase in tail-fin size was not interpreted as a secondary sexual character under the influence of the gonad since it can be produced with *Phyone* injections in castrates. These results have been confirmed by Dawson and Jimenez (1933) who, in addition, demonstrated that the hedonic glands responded in the manner characteristic of secondary sexual characters. Similar logic has been applied by Adams (1931) in a study of the effect of intramuscular injections of anuran pars anterior in immature *T. cristatus*. Here dorsoventral expansion of the tail and head enlargement were produced in both normal and castrated animals.

## MATERIALS AND METHODS

The femoral pad is of value in experimental studies as an indicator of the hypophysis-gonad-reproductive tract inter-relationship. The writer was attracted to this area as a useful indicator for studies on the

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### Explanation of Figures

1. Ventral view of an animal in which its left femoral pad has been exchanged with the skin of the pectoral area (autoplastic series) showing hypertrophy and pigmentation of normal and transplanted tissue following six injections of pituitary extract. Photograph taken three hundred and thirty-five days after graft was made.
2. Pectoral area of same animal at higher magnification.
3. Ventral view of a newt in which a femoral pad was grafted from another animal of the same species to the pectoral region (homioplastic series). Typical response of secondary sexual characters was obtained for the normal host tissue following seven doses of pituitary extract. The homioplastic transplant shows no corrugation or pigmentation. Photograph taken sixty-six days after transplantation.
4. Pectoral area of same animal at higher magnification.



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PLATE I

persistence of skin transplants. The general technique employed in this work consisted in cutting out the entire femoral area from one limb of an animal under chloretone anaesthesia and transplanting it to the midventral line between the forelimbs. Upon stimulation with intraperitoneal implants of frog anterior pituitary or an extract of whole sheep gland it was possible to determine whether the grafted tissue had persisted by referring to the pad of the uninjured leg. Only sexually mature aquatic male newts were used. All experimental animals were kept in aquaria at room temperature.

### RESULTS

Autoplastic transplants were consistently found to persist unchanged in eight animals after more than three hundred days. Figures 1 and 2 illustrate the results obtained in a typical case three hundred and thirty-five days after autotransplantation. Stimulation of secondary sexual characters was brought about by means of six doses of pituitary extract (whole sheep gland, Parke Davis and Company). Each dose consisted of an intraperitoneal injection of 0.2 cc. of a 1-5 aqueous solution. Injections were made every second day. Adequate response is evidenced (Fig. 1) by the development of hypertrophy and pigmentation of toe pads and the normal (right) femoral area. The left femoral pad is seen in the pectoral position and this appears typical (Fig. 2).

In eight cases of homoiotransplantation the femoral pad was never found to persist unchanged for more than sixty days. Progressive removal or rearrangement of grafted tissue was evidenced by the failure of characteristic hypertrophy of the transplanted pad tissue following pituitary stimulation. Figures 3 and 4 illustrate a typical

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#### Explanation of Figures

5. Ventral view of an animal one hundred and fifty days after amputation of left limb. An autoplastic transplant of the femoral pad was made at the time of amputation. Graft shows a reaction similar to the normal right leg which serves as control to the action of seven doses of pituitary extract.

6. Ventral view of an animal seventy days after amputation of the right limb. An homoioplastic transplant of femoral pad tissue was made at the time of amputation. The normal left leg shows characteristic reaction to seven doses of pituitary extract but the homoiotransplant seems almost entirely resorbed.

7. View of the ventral aspect of the pelvic area and left limb regeneration seventy days after amputation. The first toe shows definite development of pigmentation in response to seven injections of pituitary extract.

8. Ventral aspect of regenerated limb three hundred days after amputation. The form of the toes is not typical. Pigment pads have developed on all toes and there is evidence of scattered masses of pad tissue along the post-axial margin near the base of the limb.



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PLATE II

homoiotransplant in which the secondary sexual areas of the host were undisturbed and have given characteristic reactions following seven doses of pituitary extract. Photographic study at higher magnification (Fig. 4) of the pectoral graft shows complete absence of pigmented tissue.

Both auto- and homoiotransplantations have been performed on a series of animals in which limb regeneration was being studied. Confirmation of the results reported above was obtained (Figs. 5 and 6), thus excluding the possibility that lack of persistence of homoiotransplants might be due to the presence of pad tissue in excess of the normal amount.

Femoral pads were never observed to regenerate following extirpation. Denuded femoral areas to which skin from the pectoral region was transplanted failed to organize nuptial pads after more than three hundred days (Fig. 1).

Following amputation, limbs were found to regenerate but in no case were they found to equal the normal limb length nor were the relative length and form of the toes typical. These results confirm those reported by Collins (1932).

During regeneration the nuptial tissue was found to appear first in the toe pads. A definite, pigmented area was observed on the first toe tip seventy days after amputation (Fig. 7). Toe pigmentation then proceeded serially in a post-axial direction. Definite pigmentation in the femoral area was first observed at the end of the three-hundredth day of limb regeneration (Fig. 8). It was not possible to determine whether this process extended proximo-distally or disto-proximally.

#### SUMMARY

The femoral pads of the newt, *Triturus viridescens*, respond to pituitary extract stimulation by characteristic hypertrophy and pigmentation. Autoplastic transplants of pads were found to persist intact after more than three hundred days while homoiothetic transplants lost the capacity to exhibit normal reactions after sixty days. This is not a function of the total amount of pad tissue present. Regeneration of the femoral pads in denuded areas was never observed. Regenerated limbs showed pigmentation of the first toe within seventy days. Evidence of the reorganization of the femoral pad was noted three hundred days after amputation.

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